

Role of hydrophobic and electrostatic interactions for initial enteric virus retention by MF membranes

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Abstract

Membranes are of increasing interest for the removal of human enteric viruses from wastewater, especially when the goal of treatment is reuse. Limited work has been undertaken on fundamental issues such as aggregation and the role of electrostatic and hydrophobic interactions, as opposed to the sieving of viruses by membranes. One apparently critical factor would be the iso-electric point (pI) of a virus. As an example of a worst-case model virus, the retention of bacteriophage MS-2 was investigated using hydrophobic (GVHP) and hydrophilic (GVWP) 0.22 µm MF membranes at different pH levels and with different salts. High retention levels were measured at the iso-electric point of MS-2, pH 3.9 (5 log retention) and pH 7 (4.3 log retention) in the presence of salts and with a hydrophobic membrane. When retention was compared on a hydrophilic membrane, it was clear that hydrophobic interactions dominated virus retention, and this was improved by salt, presumably causing reduction of the Gouy double-layer when MS-2 was charged (pH 7). This paper shows that knowledge of the adsorption characteristics of viruses and the suspending conditions are important to predict removal of viruses by hydrophobic MF membranes, and discusses some of the practical implications of these important hydrophobic interactions.

Keywords: Microfiltration; Adsorption; Hydrophobicity, Water Treatment

1. Introduction

In recent years the reuse of wastewater for non-potable use has gained much attention. The presence of human enteric viruses is a major risk associated with wastewater reuse [1-4]. Therefore, enteric virus removal requires specific attention, given their low infection dose, long-term survival in the environment and low removal efficiency in conventional wastewater treatment [5]. Detection and enumeration of human enteric viruses is expensive and time consuming, hence bacteriophages such as the F-RNA coliphages have been suggested as useful models because of their similar size and survival in waters [6]. MS-2 is the most studied F-RNA coliphage [6], and given its low iso-electric point (pI=3.9) [7] and relative hydrophobicity [8] it makes a good worst-case strain for membrane interaction studies. The main characteristics of some human enteric viruses and bacteriophages are summarized in Table 1.

Retention of the F-RNA coliphage, Qβ, using a new MF membrane with a pore size of 0.1 µm has been reported at about 90% [5]. Removal was increased, however to 99.5% in the presence of particulates (from pond water and activated sludge). Similarly, Otaki et al. [9] reported 40-90% removal of F-RNA coliphages to *E. coli* K12, using a MF membrane with a pore size of 0.2 µm. Removal was increased to 88-99% with a 0.1 µm pore-size MF membrane, despite the significantly smaller diameter of the coliphages (~24 nm). The coliphage findings appear similar to reported poliovirus removal, where up to 99% removal was observed with 0.2 µm MF membrane [10]. In contrast to the retentions on standard hydrophobic MF membranes, Herath et al. [5] reported only 35-80% removal of Qβ and 30-85% of MS-2 coliphages using 50 nm hydrophilic nucleopore membranes at various pH levels. At the pH's around the pI of MS-2 and Qβ, the highest retention was reported and it was suggested that aggregation enhanced retention, even below their pI values [5].

Besides the use of MF membranes, the smaller pore size ultra-filtration (UF) membranes have been used for the removal of enteric viruses and not surprisingly high removal efficiencies have been achieved [10]. Nonetheless, despite the relatively large pore sizes of MF membranes (0.22-0.45 µm) for the removal of small viruses like Hepatitis A (27 nm) and Norwalk-like viruses (35-39 nm), MF membranes appear to be capable of high removal efficiencies [11-13]. In addition, there is evidence that pore size alone does not adequately describe the ability of a filter to remove particulates from solutions [14]. For example, the important factors for adsorption of viruses to membranes are the chemical composition of the membrane, the ratio of membrane pore diameter to virus diameter and hydrophobic and electrostatic interactions [10]. In previous research the ratio of membrane pore diameter (0.22 µm) and virus diameter (MS-2, 25 nm) was one of the major areas of interest regarding the retention of viruses by MF and UF [15, 16].

Very little work has been undertaken on the importance of electrostatic and hydrophobic interactions in virus removal by membranes. One apparently critical factor would be the iso-electric point (pI) of a virus and this knowledge makes it possible to predict the likelihood of its attachment to a charged surface [17]. The charge of most viruses will be negative under the conditions present in most wastewater effluents (i.e. pH 6-7). The net neutral charged at a virus's pI leads to maximum virus-virus coagulation [5]. Aggregation may therefore further promote virus retention by membranes. It has also been reported that the presence of particular ions promotes virus aggregation compared with buffers at low pH alone [18].

For electrostatic interactions of viruses, the thickness of the double layer as described by Gerba [8] (see Figure 1) plays the most important role. In Figure 1 the solid could represent the membrane surface and it should be noted that the concentration in the boundary layer of the membrane could differ to the concentration present in the bulk solution. Both the pH and the presence of salts (in the

bulk solution) influence the thickness of the double layer. Increasing the salt concentration or valency reduces the thickness of this layer and facilitates virus adsorption to surfaces [8].

Hydrophobic interactions between viruses and surfaces may also contribute significantly to adsorption [17]. Due to the increased electrostatic repulsion at higher pH levels, hydrophobic interaction could play the major role in maintaining virus-filter adsorption [8]. Gerba goes on to conclude that some salts will have a positive effect on the adsorption of viruses to surfaces, by increasing the ordering of water molecules and promote the sequestering of hydrophobic entities [8]. Nonetheless, these findings are mostly based on adsorption of viruses to soil or sand, little is known about adsorption of viruses to membranes. Therefore, the aim of this paper was to describe the adsorption interactions, electrostatic or hydrophobic of MS-2 to MF membranes during the first stage of filtration. The adsorption of MS-2 to the membrane was investigated at different pH levels, with different salts and with hydrophobic and hydrophilic membranes using different test volumes. Before starting these experiments the effect of using stirring in the dead-end membrane experimental unit and that of MS-2 aggregation at different pH levels were determined. A final experiment was performed to see the influence of time and permeate volume on the retention of MS-2 by MF membranes.

2. Experimental

2.1 Membranes

Hydrophobic (GVHP) and hydrophilic (GVWP) MF membranes with a nominal pore size of 0.22 μm (Millipore, Australia) were used in this study. The hydrophilic membrane was a modified hydrophobic membrane. The membrane material was a modified polyvinylidene fluoride (PVDF). The membranes were negatively charged over most of the relevant pH range (3-7), as illustrated in Figure 3, where the zeta potential is given as a function of the pH, for both the GVHP and GVWP membranes, submitted from [21]. The measured flux for both GVHP and GVWP membranes were $3822 \pm 304 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ and $3762 \pm 241 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, respectively. These fluxes were obtained at a temperature of 10-15 $^{\circ}\text{C}$.

2.2 MS-2 Coliphage

Concentrations of infective MS-2 particles were determined by the plaque forming unit (pfu) assay, using the double agar overlay (DAL) method and *Salmonella typhimurium* WG49 as the host bacterium [19]. MS-2 was harvested off 24h cultures from agar plates, 0.22 μm filtered and the concentrated stock (about $10^{12} \text{ pfu}\cdot\text{mL}^{-1}$) stored in MilliQ water at 4 $^{\circ}\text{C}$ for up to 40 days.

2.3 Aggregation test

Citrate phosphate and NaHCO_3 buffers were used to determine the effect of pH on MS-2 aggregation. The citrate phosphate buffer was used for the wide pH applicable, the NaHCO_3 buffer was used as the buffer present in natural waters. For the citrate phosphate buffer, 0.1 M citric acid and 0.2 M Na_2HPO_4 were added to provide pH 3.9 and 7.0 [20]. The NaHCO_3 buffer was made to a concentration of 1 mM and the pH was adjusted with 0.5 M HCl or 0.5 M NaOH solutions. The test volume was 100 mL and MS-2 was added to a final concentration of $1\cdot 10^2 \text{ pfu}\cdot\text{mL}^{-1}$. A sample was taken in triplicate every two hours using the citrate phosphate buffer and every three hours using the NaHCO_3 buffer.

2.4 Filtration procedure

All experiments were carried out in a magnetically stirred batch cell (volume 110 mL, membrane area 15.2 cm^2) at a pressure of 50 kPa, pressurized with nitrogen gas. A reservoir of 1.5 L was

connected to the stirred cell (Figure 2). During all experiments the flux was measured using an electronic balance.

The hydrophobic membrane was soaked in 50% ethanol for 10 minutes to wet the pores and then rinsed with MilliQ water. Before the start of an experiment with the virus suspension, the pure water flux was determined by using 1L of MilliQ.

For the stirring effect the speed of the stirrer was adjusted to 270 rpm and the feed volume added to the stirred cell was 100 mL NaHCO_3 buffer, pH was adjusted by adding 0.5 M HCl. Initial phage concentration was $1\cdot 10^4 \text{ pfu}\cdot\text{mL}^{-1}$. This concentration represents the average concentrations of coliphages found in secondary effluent.

For the adsorption experiments different test volumes were used. A volume of 10 mL at pH 3.9 or 7 of feed solution was poured into the stirred cell. Using this volume the influence of the hydrophobicity of a membrane and salts were investigated. A test volume of 100 mL was used for all pH levels (3.9, 5, 6, 7) with and without the presence of salts, but only tested with the GVHP membrane. Concentrated virus stock was diluted to yield $1\cdot 10^6 \text{ pfu}\cdot\text{mL}^{-1}$ in NaHCO_3 buffer (1.0 mM). The pH was adjusted by adding 0.5 M HCl. For the salt experiments, NaCl was added to give a concentration of 0.02 M and CaCl_2 to 0.5 mM. These concentrations were recommended by Schäfer [21] and reflect the concentrations in natural waters. After the virus experiment the pure water flux was determined again with 1 L of MilliQ. The concentration of MS-2 in the feed and permeate were measured in duplicate for the stirring experiment and in triplicate for the adsorption and long-term experiment. All concentrations were expressed in $\text{pfu}\cdot\text{mL}^{-1}$.

For the longer-term experiment, the feed volume was 2 L instead of 10 mL or 100 mL, with an average phage concentration of $1\cdot 10^3 \text{ pfu}\cdot\text{mL}^{-1}$. The feed was added in two separate liters into the feed cell, without disturbing the flux measurement. The permeate concentration was determined at different time intervals in triplicate and averaged.

The retention of MS-2 was calculated with Equation 1, which gives the log reduction value (LRV), in which C_f was the feed concentration and C_p the permeate concentration.

$$\text{LRV} = \log C_f / C_p \quad (1)$$

In the long-term experiment the retention was expressed in percentages (Equation 2).

$$\text{Retention (\%)} = (1 - (C_p/C_f)) \cdot 100 \quad (2)$$

The results were analyzed using analysis of variance (ANOVA) procedure with SigmaStatV2.03 (SPSS Inc., San Rafael, CA) software. The p-values calculated in the ANOVA measures the believability of the null hypothesis. The smaller the p-values are the stronger the evidence that the groups are different.

3. Results and discussion

3.1 Aggregation test

The plaques formed with the DAL method could represent one infecting virus, or an aggregate of infecting viruses. Therefore a lower number of $\text{pfu}\cdot\text{mL}^{-1}$ could probably imply that aggregation had occurred. On the other hand at pH 3.9, inactivation could also occur, for that reason samples were taken every 2 or 3 hours for 8 and 6 hours respectively. The results obtained are shown in Figure 4. For both buffers a significant ($P < 0.001$) difference was found between pH 3.9 and 7.0, no significant difference was found between the different time samples. These results suggest that aggregation occur at the pI of MS-2 using both buffers, but further experiments over a longer time should be undertaken to confirm this. Possible interactions of MS-2 with phosphate were also taken

into account by the use of the citrate phosphate buffer. For the membrane experiments the NaHCO_3 buffer was used, because of the possible interference of phosphate with the membrane.

3.2 The effect of stirring on the adsorption of MS-2 to a GVHP MF membrane

Stirring had varied effects on retention of MS-2 with the GVHP membrane (Figure 5). At pH 3.9 there was an apparent difference between stirred and unstirred conditions (dead-end mode), but this difference was not significant ($p > 0.05$). For the other pH levels (5, 6 and 7), p-values were significant and respectively, < 0.001 , < 0.001 and 0.02. These findings may be partly explained by the results of Madaeni *et al.* [10], where higher retentions of poliovirus were reported under stirred conditions at neutral pH. It should be noticed that the pI of poliovirus 1 is 6.6 [22] but for MS-2 it is 3.9. This means that for both viruses the positive effect of stirring occurred close or at the pI of the virus.

In our study, however, the positive effect of stirring disappeared and turned into a negative effect, as the pH increased above that of the pI of MS-2. An explanation could be the fact that above the pI of a virus, hydrophobic interactions play a more important role in the adsorption to the membrane than at the pI of a virus, where both electrostatic and hydrophobic interactions could play a role in the case of MS-2. Therefore at the higher pH levels, stirring could have a negative effect on the adsorption of MS-2 to the membrane more than at the pI of MS-2, which implies that the hydrophobic interactions are probably more sensitive to stirring than the electrostatic interactions. In practical then, knowledge of the virus pI probably gives an indication if stirring (or dead-end mode) is favorable or not for higher retentions.

3.3 The effect of pH, salts and membrane hydrophobicity on the adsorption of MS-2 to a MF membrane

pH 3.9

The calculated values of the LRV for MS-2 as a function of pH, salts and different MF membranes are given in Figure 6 (test volume 10 mL), and Table 2 (test volume 100 mL). The significant differences between the presence of different salts using the two different membranes are summarized in Table 3 for the test volume of 10 mL only.

Using the unstirred cell configuration with 10 mL test volume (dead-end), the highest retention numbers occurred at pH 3.9 using the GVHP membrane (4.9 logs), and improved significantly with the presence of NaCl or CaCl_2 , which could be seen from Figure 6. Looking at Table 2 the same positive effect of salts at this pH in a 100 mL test volume was shown. Given the expected net zero charge of MS-2 at pH 3.9, but the slightly negative charge of the membrane [21], electrostatic interactions as well as hydrophobic interactions could have occurred. The increase in virus retention after adding NaCl or CaCl_2 , could be explained by the fact that the presence of certain salts have a positive effect on both interactions [8]. On the other hand no significant difference was observed after adding CaCl_2 . This was probably due to the fact that the concentration of CaCl_2 was much lower than the concentration of NaCl, to reflect the concentration of the salts in natural waters. Salt concentrations in sewage effluent would be expected to be higher, and may give higher retentions, due to the decrease of the Gouy layer (Figure 1) around the virus and thus increasing the chance of attachment to the membrane.

To largely exclude hydrophobic interactions the same experiment was conducted using the GVWP membrane (only with the test volume of 10 mL). The retention of MS-2 at pH 3.9 with the GVWP membrane decreased significantly. In contrast, a significant increase in virus retention was observed in the presence of CaCl_2 , but not in the presence of NaCl. These differences to the hydrophobic membrane may be explained by the fact that the monovalent and large radius of hydration with NaCl [22] has less influence on the electrostatic interactions, than on hydrophobic interactions. On the other side, a divalent cation, like Ca^{2+} , has a higher surface charge and therefore a smaller radius of hydration [22]. These radii of hydration determine the thickness of the Gouy layer (Figure 1)

around the virus particle and thus the ability of attachment to the membrane surface. Hence, for the GVWP membrane NaCl increased the Gouy layer and did not increase adsorption of MS-2.

It should also be noted that the CaCl_2 concentration was lower than NaCl as mentioned earlier. The lower retentions of MS-2 measured using the GVWP membrane, compared to the GVHP membrane, was probably due to the fact that even at the pI of the virus, hydrophobic interactions played the major role, which would be absent with a hydrophilic membrane. Herath *et al.* [5] reported up to 80% retention of MS-2 at pH 3.9 and 20% at pH 7, using hydrophilic MF membranes with a pore size of 50 nm. The reported retention was higher than reported in the current research (50% versus 8% respectively). This difference could be explained by the fact that the pore size was ten times larger than MS-2 (25 nm) compared to just two-times larger in Herath's [5] research. This implies that the chance of adsorption (due to electrostatic interactions) was larger with the smaller pore-size membrane, and thus higher retention levels could be achieved. Furthermore, the larger size of an aggregate would have caused the higher rejection in Herath's research [5]. On the other hand the particle/pore size ratio of 1:2 could cause high rejection due to steric effects. Aggregation may have played a role in the current study, but on the other hand, the hydrophobic and electrostatic interactions appear to play a more important role.

pH 7

The retentions measured for MS-2 at neutral pH (7.0) were lower than at pH 3.9, which could be seen from Figure 6. At a pH of 7.0, both the virus and the membrane would be negatively charged. From this it could be expected that hydrophobic interactions were probably the main interactions for adsorption to the GVHP membrane and thus responsible for the retention of MS-2 at this pH. In addition to limited electrostatic interactions, a possible explanation for the difference in retention at pH 3.9 versus 7.0 could be the difference in the hydrophobicity of MS-2 and the membrane at the different pH levels. These factors would have resulted in difference hydrophobic interactions with the membrane. The same positive effect for both NaCl and CaCl_2 , seen at pH 3.9 were present at pH 7.0, using the GVHP membrane. With NaCl, the retention was almost doubled that of the no salt situation. Hence, it is likely that both NaCl and CaCl_2 influenced hydrophobic interactions in a positive way at pH 7.0, and thus improves the retention of MS-2.

The results obtained at pH 5 and 6 (Table 2) are quite similar with each other. Comparing the retention of MS-2 at pH 7 without any salts it is seen that the retention numbers at pH 5 and 6 are higher. This could be explained by the fact that at pH 5 and 6 the virus is negatively charged, but less than at pH 7, which, together with the probable changes in hydrophobicity of both the membrane and the virus, could explain the higher retention numbers obtained at these pH levels. The positive effect of salts on the retention of MS-2 at these pH levels, confirm the results found at pH 3.9 and 7.

To see if there were any electrostatic interactions possible at pH 7 the same experiments were repeated using the hydrophilic membrane (only using a 10 mL test volume). In the absence of hydrophobic interactions, the retention dropped to almost zero (8 and 18%). Therefore, it appears that electrostatic interactions play a minor role in the adsorption of MS-2 at pH 7.0 to the membrane, and thus to retention efficiency. No significant effect of salts was observed, at this low level of retention.

From the results obtained at pH 3.9 and 7.0 using the two different membranes, it became clear that a higher initial retention was achieved using the hydrophobic membrane and that hydrophobic interactions played the major role in the retention. These results concur with those obtained at pH 5 and 6. Bales *et al.* [23] also concluded that hydrophobic effects are important for adsorption of even relatively hydrophilic viruses. Furthermore, knowledge about the pI of a virus and, is a factor regarding the removal of viruses by MF membranes. As stated by Gerba [8], the knowledge of the pI of a virus makes it possible to predict the likelihood of its adsorption to a charged surface, as

long as the suspending conditions are known. Dowd [7] concluded that the pI of a virus is the predetermining factor controlling viral adsorption within aquifers. Besides the adsorption characteristics, also the effect of stirring appears to depend on the pI of the virus. On the other hand, it is well understood that the pI of viruses could even differ within the same strain [8]. The difference in pI is mainly caused by the fact that different viruses have different protein coatings (capsids) that surround the virus. Besides that, metals and other substances present in water could form complexes with the capsids, which will have an impact on adsorption characteristics and the measured pI.

Given the pI of MS-2 of 3.9 versus the pI of various enteric viruses such as Norwalk (pI 5-6), MS-2 would not be expected to mimic the subsurface filtration of Norwalk virus in natural systems, as reported by Redman *et al.* [24]. Hence, MS-2 would be less "sticky" and therefore may represent a worst-case model. Therefore, to predict removal of viruses by membranes a combination of viruses may be considered that represents a range of adsorption characteristics [17]. Alternatively, a virus that adsorbs less than other viruses under certain conditions may be considered as a worst-case model virus [17]. Viruses with a strong negative surface charge and little hydrophobicity meet these requirements [17].

3.4 Longer term experiment

Initial virus adsorption to clean, new membranes was the focus of this research. It is important to note that by increasing the filtrate volume virus retention decreased (Figure 7). The overall retention was calculated from samples out of the 2 L permeate. The membrane used was the GVHP and the pH applied was 3.9. During the first 0.5 L (~6 minutes), the retention achieved was similar to that described in the earlier experiments with 10 mL. After approximately 1 L (~11 minutes), the retention started to decline. What probably happened was that after a while the most accessible adsorption sites have been accommodated [10]. However, Madaeni *et al.* [10] showed that retention gradually increased again with further volumes filtered. Such a rise was not measured in the current experiment, possibly due to insufficient volume being processed (two rather than the seven liters used by Madaeni *et al.* [10], using the same types of membranes).

Hence, in practice there may be two opposing reactions after membrane cleaning or backwash. On the one hand increasing virus breakthrough due to increasing volume being processed, versus increased membrane fouling and associated entrapment of viruses. The presence of organics in wastewaters could also compete for adsorption sites, and provide hydrophobic adsorption sites [17]. Hence, future studies will investigate these possible interactions.

4. Conclusions

From the initial membrane experiments it was observed that above the pI of MS-2, stirring had a negative effect on the retention of MS-2 using MF membranes. At the pI (3.9) of MS-2 no significant difference was observed between stirred and unstirred conditions, as hydrophobic interactions appeared to dominate.

Using MF membranes with a nominal pore size of 0.22 µm, the highest retentions were achieved using a hydrophobic membrane at pH 3.9 in the presence of 0.02 M NaCl (5.9 log retention). Even at a pH of 7.0, high retentions were achieved in the presence of NaCl or CaCl₂ (4.3 and 2.9 log retention respectively), with the hydrophobic membrane. Poorer retentions were observed using a hydrophilic membrane (0.3 log retention at pH 3.9 and 0.04 at pH 7).

The presence of NaCl or CaCl₂ had positive effects on hydrophobic interactions, which was observed from the experiments at pH 5.6 and 7 using the hydrophobic membrane.

Hydrophobic interactions appear to play the major role in the retention of MS-2. The electrostatic interactions were playing a role at pH 3.9, but were probably less important than the hydrophobic interactions.

The results of the aggregation test indicated MS-2 aggregation at pH 3.9, but it was hard to say if aggregation was also partly responsible for the level of membrane retention at this pH.

Given the effect of stirring and the adsorption characteristics of a virus, it is important to know the pI of the viruses of concern. Nonetheless, given that most human enteric viruses have pI's >3.9 [25], MS-2 should be considered an appropriate worst-case model virus for membrane studies. The initial adsorption effects observed in this paper show that the hydrophobic membrane has an adsorption capacity. This also implies that the hydrophobic membrane is more likely to cause a bulk release of viruses if conditions vary, which may be of health concern. Further research should be undertaken on the long-term performance of membranes and the effects of organics present in treated wastewater or use of additives to improve virus retention.

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Table 1 Main characteristics of two human enteric viruses and the F-RNA coliphages MS-2 and Qβ

Virus	Family/Group	Particle morphology	Genome	Size(nm)	pI (-)
MS2	<i>Leviviridae</i> (F-RNA coliphage)	Symmetry icosahedral	Linear positive-sense s/s RNA	23-25	3.9 ⁽¹⁾
Qβ	<i>Leviviridae</i> (F-RNA coliphage)	Symmetry icosahedral	Linear positive-sense s/s RNA	24-25	5.3 ⁽¹⁾
Hepatitis A	<i>Picornaviridae</i>	Symmetry icosahedral	Linear positive-sense s/s RNA	27	unknown
Norwalk	<i>Caliciviridae</i>	Symmetry icosahedral	Linear positive-sense s/s RNA	35-39	unknown

⁽¹⁾ From [7]

Table 2 Effect of pH, salts and membranes on log removal (LRV) of MS-2 (initial concentration 10⁶ pfu·mL⁻¹ in 100 mL) using GVHP microfiltration membranes

Salt	pH	3.9	5	6	7
None		4.0	0.98	1.0	0.3
NaCl (0.02 M)		>6	3.7	3.1	3.4
CaCl ₂ (0.5 mM)		5.0	2.4	2.5	1.9

Table 3 P-values for comparisons of salt treatments at different pH, using a GVHP and GVWP membrane within a test volume of 10 mL

Pair	Membrane	GVHP		GVWP	
		pH 3.9	pH 7	pH 3.9	pH 7
NaCl v no salts		0.001	0.001	0.652	0.155
CaCl ₂ v no salts		0.477	0.001	0.004	0.155
NaCl v CaCl ₂		0.002	0.971	0.001	0.155

(p-values < 0.05 means that there is a significant difference)

Figure Captions

Figure 1 Overview virus structure presentation of the double layers, adapted from Gerba [8]

Figure 2. Dead-end membrane stirred cell with 1-L feed unit (right) and balance to weigh permeate (foreground). Adapted from [21]

Figure 3 Zeta potential of clean membranes (a) GVHP, (b) GVWP, (c) GVHP pretreated with 50% ethanol solution. Adapted from [21]

Figure 4 MS-2 concentration (pfu·mL⁻¹) as a function of time in a citrate phosphate buffer (CP) and a NaHCO₃ buffer at pH 3.9 and 7

Figure 5 Retention profiles of MS-2 (initial concentration 10⁴ pfu·mL⁻¹) under stirred (270 rpm) and unstirred conditions, using GVHP membranes

Figure 6 Retention profiles of MS-2 (initial value 10⁶ pfu·mL⁻¹ within 10 mL test volume) at different conditions under unstirred conditions, using GVHP and GVWP membranes

Figure 7 Retention profile of MS-2 (initial value 10³ pfu·mL⁻¹) at pH 3.9 using the GVHP membrane under unstirred conditions, as a function of the permeate volume. The grey square represents the retention with the GVWP membrane at pH 3.9 after 10 mL

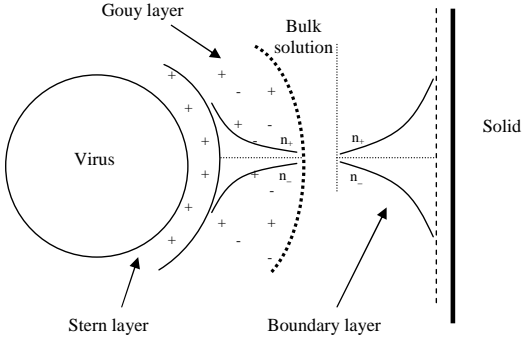


Figure 1

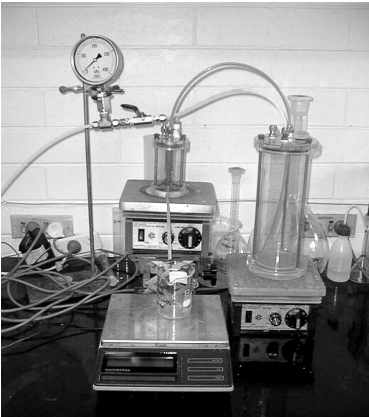


Figure 2

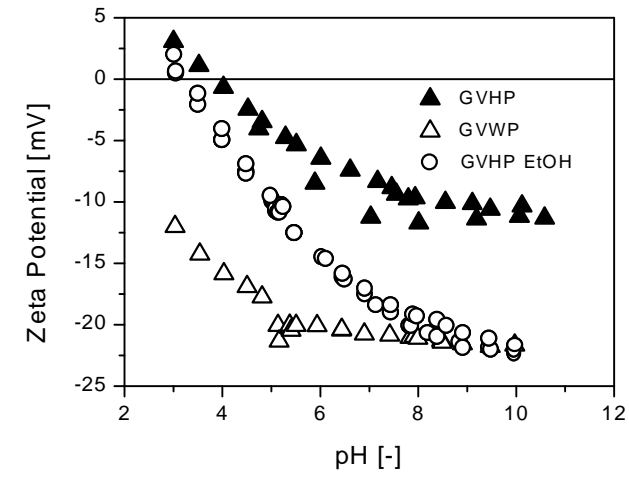


Figure 3

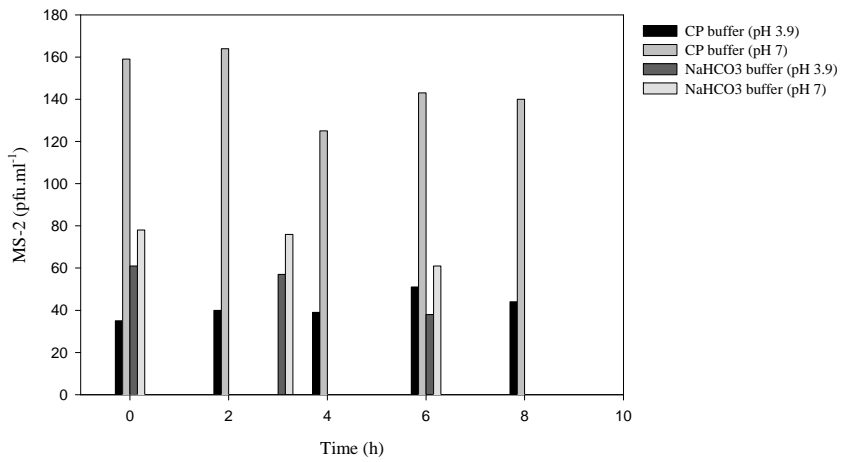


Figure 4

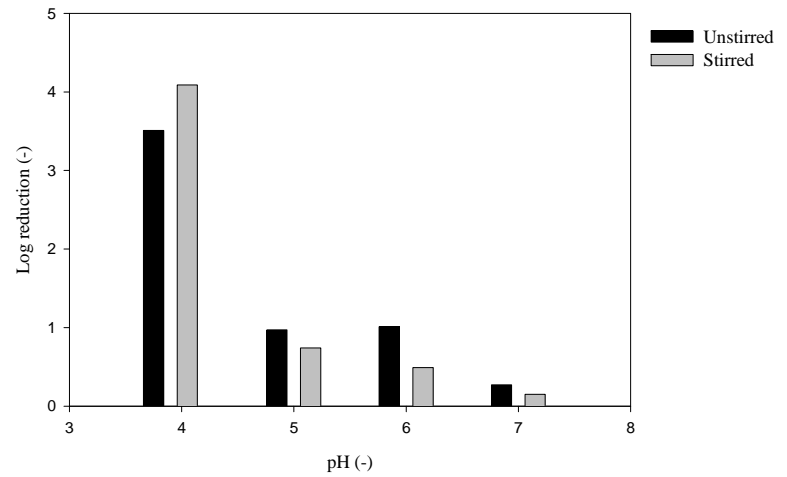


Figure 5

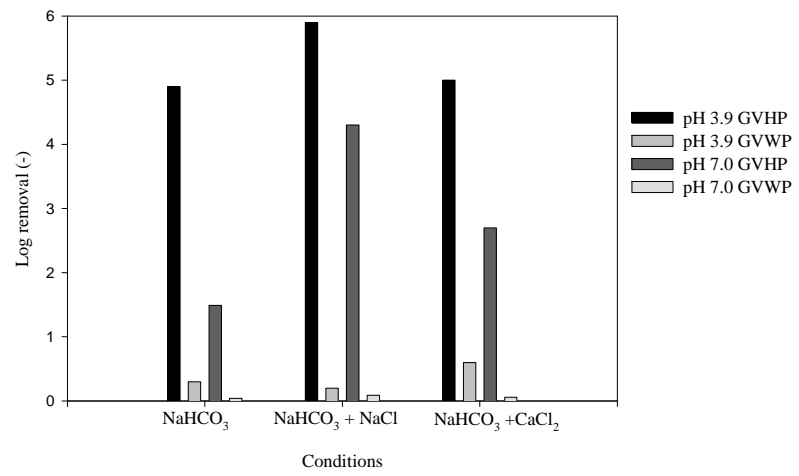


Figure 6

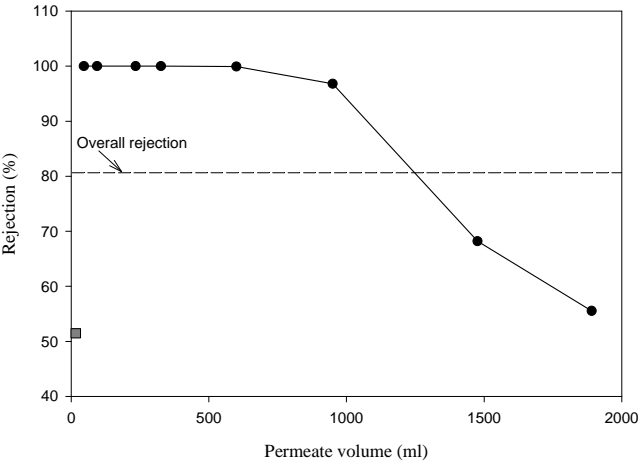


Figure 7